Supporting Information

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SI Discussion

The regulatory states of the mixed phenotypes may be further explained by changes in expression of signaling receptors related to cell migration. In the sea urchin, Vegf ligand is expressed in two areas of mediolateral ectodermal cells and instructs skeletogenic micromere (SM) cells, which exclusively express the Vegf receptor, to cluster into two subepithelial regions. Inhibition of Vegf signaling causes incorrect positioning of SM cells and a disruption of the skeletogenic program (1). *Gcm* overexpression represses VegfrII expression (Fig. S5*A*), along with that of other skeletogenic genes, and explains why SM cells expressing the *tbr:*GCM BAC would have reduced sensitivity to the ectodermal Vegf signal. The ability of *gcm* overexpression to lock out the SM cell response to Vegf signaling might be what permits the alternative migration patterns of the transformed cells. FGF signaling also plays an instructive role in SM migration patterns and in skeletogenesis (2), and FGF receptor expression is also likely downregulated in respecified *tbr*:GCM-expressing cells. Although the signaling factors that guide pigment cell migration to the aboral ectoderm have not yet been elucidated, they may play complementary roles in permitting the proper migration of converted or mixed-fate SM cells seen in our experiments.

SI Materials and Methods

Cloning of Tbr-BAC GFP Reporter and Tbr-BAC GCM Expression Construct. A 138-kb BAC clone Sp_031J08_L was identified from a *Strongylocentrotus purpuratus* genomic DNA library from the Sea Urchin Genome Resource (Andy Cameron; http://sugp.caltech.edu). This clone contains the entire Tbr-coding sequence, and the start site of transcription is flanked by an at least 60-kb genomic sequence on each side. The full-length spGCM cDNA clone 4I5 was isolated from a 15-h *S. purpuratus* cDNA library. It contains the complete spGCM-coding sequence and 3′-UTR. Recombinant BAC cloning was used to generate Tbr-GCM/GFP expression constructs as described (3). GFP- or spGCM-coding sequence was cloned into a vector upstream of a kanamycin-resistance gene flanked by flp recombinase sites. Recombination target sequences, roughly 150 bp in length on each end, were cloned upstream of the GCM- or GFP-coding sequence and downstream of the kanamycin resistance marker. Homologous recombination was used to replace 150 bp of Tbr sequence (40 bp of 5′-UTR and 110 bp of coding sequence) with the coding sequence and 3′-UTR of spGCM. This construct was called the Tbr::Gcm BAC. A Tbr:GFP BAC was similarly constructed using GFP-coding sequence containing an SV40 3′-UTR polyadenylation tail and was coinjected with Tbr::Gcm BAC, which acts as a reporter of exogenous Gcm expression. Ets1:GFP BAC and Alx:GFP BACs were constructed in the same manner.

The upstream recombination target sequence on the Tbr BAC was TTTCGGAAAAAGTGTTAAAATCGCAGTGAGAATTTCATCAGCGTTCGCGCCTTCTCGCTTCTGTGTTTATCCATGTAATTTGTGACTGAATTTTCGCACTCCGACTCTAACCCTAATTTAAAGGGATTGAATTCTAACGCCTTCGCGC. The downstream target sequence on the Tbr BAC was TGAAGATGAGAATCTTGATAGAGATGACGGGAGCAATGGATCTGAAGATACCAACTGCGAAAAGTCAACAGTCGAACAATTTCACACCAATAAATTAATTTCAAACGCTGATCATAACGTCGGGGATCCAAATAACGACTACCCTTGC.

Whole-Mount in Situ Hybridization. Single- and two-color whole-mount in situ hybridization (WMISH) were performed as described by (4) (wnt8 inputs paper) for detection of Digoxygenin (DIG)- and Dinitrophenol (DNP)-labeled probes. In two-color WMISH, DNP-labeled probes were detected with nitro-blue tetrazolium/5-bromo-4-chloro-3′-indolyphosphate (NBT/BCIP)-staining solution. The reaction was stopped by washing with Mops buffer, and alkaline phosphatase activity of the anti-DIG-AP Fab fragments was inactivated by glycine-HCl treatment. A second stain was performed on DIG-labeled probes using 2-(4-iodophenyl)-5-(4-nitrophenyl)-3-phenyltetrazolium/BCIP (INT/BCIP) staining solution. Embryos were finally transferred to 70% glycerol and imaged.

Microinjections. ***Tbr-GCM and Tbr-GFP BAC constructs.*** BAC DNA constructs were linearized using the homing endonuclease, PI-SceI to produce 140-kb fragments. A single PI-SceI exists on the pBace3.6 vector used to construct the *purpuratus* genomic BAC DNA library. BAC DNA was injected into fertilized eggs as described previously (5) except that no carrier genomic DNA was used. Injection volumes were in the range of 5–10 pL and at a concentration of 500 copies per picoliter.

Pks-GFP construct. A reporter construct driving pigment-cell-specific expression of dsRed was cloned. The reporter contains 2.0 kb of genomic DNA upstream of the start site of SpPks transcription and includes the basal promoter and a proximal *cis*-regulatory module that is capable of recapitulating endogenous Pks expression at 24 and 48 h post fertilization (6). The primers used to amplify the pks genomic DNA were an upstream primer (5′-TCCCTCTTTCTCTCCCACTCTC-3′) and a downstream primer (5′-CTCTGTTTCTTGCTACAACTCTC-3′).

Short Tbr-GCM/GFP constructs. Five-kilobase Tbr constructs containing all of the *cis*-regulatory information necessary to recapitulate embryonic Tbr expression were PCR-amplified from the BAC-GFP and BAC-GCM constructs (left primer: 5′-TCGGAACGATACGAAAACTTTG-3′; right primer: 5′-ACTGCCTCCCTGTTTGAGAA-3′).

Small constructs were injected as described (5). Injection volumes were in the range of 5–10 pL and at a concentration of 1,000 copies per picoliter. mRNA injection volumes were also 5–10 pL but at a concentration of 1 μg/μL. The copy number of injected mRNA was determined after injection by quantitative PCR.

1. Duloquin L, Lhomond G, Gache C (2007) Localized VEGF signaling from ectoderm to mesenchyme cells controls morphogenesis of the sea urchin embryo skeleton. *Development* 134:2293–2302.
2. Röttinger E, et al. (2008) FGF signals guide migration of mesenchymal cells, control skeletal morphogenesis [corrected] and regulate gastrulation during sea urchin development. *Development* 135:353–365.
3. Sweet HC, Hodor PG, Ettensohn CA (1999) The role of micromere signaling in Notch activation and mesoderm specification during sea urchin embryogenesis. *Development* 126:5255–5265.
4. Minokawa T, Wikramanayake AH, Davidson EH (2005) Cis-regulatory inputs of the wnt8 gene in the sea urchin endomesoderm network. *Dev Biol* 288:545–558.
5. Wahl ME, Hahn J, Gora K, Davidson EH, Oliveri P (2009) The cis-regulatory system of the tbrain gene: Alternative use of multiple modules to promote skeletogenic expression in the sea urchin embryo. *Dev Biol* 335:428–441.
6. Damle S, Davidson EH (2011) Precise cis-regulatory control of spatial and temporal expression of the alx-1 gene in the skeletogenic lineage of S. purpuratus. *Dev Biol* 357:505–517.

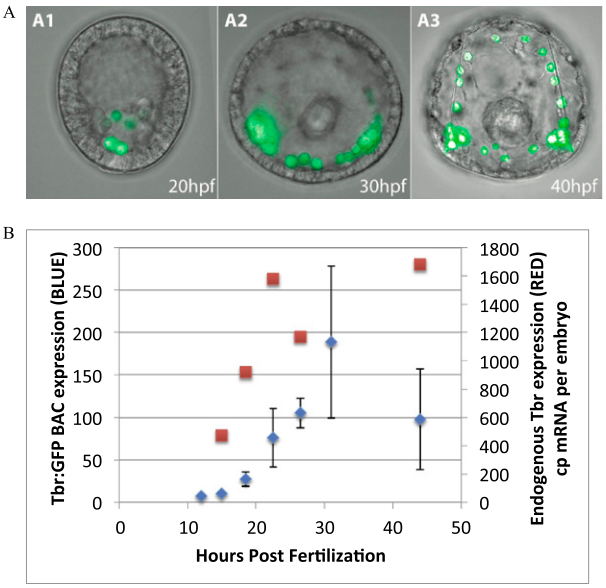

Fig. S1. Expression of GFP reporter driven by Tbr BAC. (*A*) GFP fluorescence in embryos injected with the Tbr::GFP BAC reporter construct at 20 (*A1*), 30 (*A2*), and 40 (*A3*) hours post fertilization (hpf). GFP expression at 20 hpf shows mosaic incorporation characteristic of DNA reporter injections. At 30 hpf, primary mesenchyme cells form a syncytium, leading to the even distribution of GFP across all fused cells. (*B*) Endogenous Tbr and GFP transcript abundance, measured by quantitative PCR. The copy number of transcripts was calculated by comparing against simultaneously measured ubiquitin mRNA. Ubiquitin has an abundance of roughly 88,000 subunit transcripts per embryo. $n = 3$ with respect to Tbr:GFP BAC injection data.

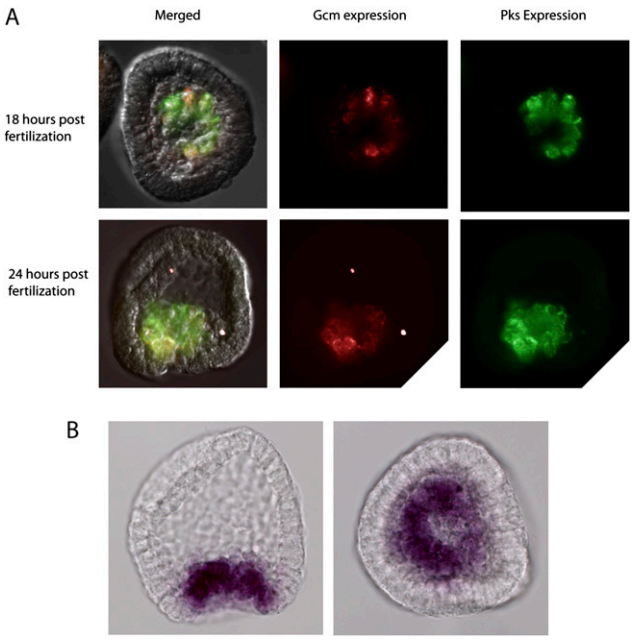

Fig. S2. Expression of *gcm*, *pks* and *fmo* genes. (*A*) Two-color fluorescent whole-mount in situ hybridization was performed using DIG-conjugated antisense probe against gcm and a DNP-conjugated pks probe at 18 and 24 hpf. The 18-hpf embryo is rotated to the vegetal pole to show staining in a half-ring of NSM that marks cells fated to become pigment cells. Embryos at 24 hpf are oriented laterally and show staining of ingressed pigment cell precursors. (*B*) Lateral and vegetal view of fmo expression in 24-hpf embryos.

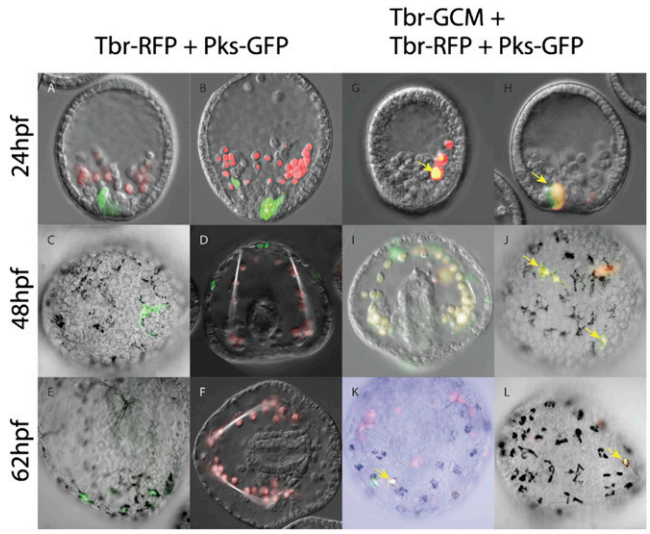

Fig. S3. *tbr*:RFP and *pks*:GFP small-construct reporters were cloned such that their expression matches the expression of endogenous *tbr* and *pks*. These constructs were coinjected either in the presence or the absence of a *tbr*:GCM small construct and observed during development. In *A–F*, embryos were co-injected with only *tbr*:RFP and *pks*:GFP. (*A*, *C*, and *E*) Expression of *pks*:GFP reporter in SM cells and eventually in pigment cells in the aboral ectoderm. (*B*, *D*, and *F*) Expression of *tbr*:RFP construct in ingressing SM and eventually in the skeleton. (*G–L*) Embryos were coinjected with the *tbr*:GCM as well as *tbr*:RFP and *pks*:GFP. (*G* and *I*) Coexpression of RFP and GFP in Primary Mesenchyme Cells (PMCs) and skeletogenic cells. (*H*, *J*, *K*, *L*) Coexpression of RFP and GFP in SM cells and pigment cells. Yellow arrow indicates coexpression of *pks*:GFP and *tbr*:RFP small-construct reporters.

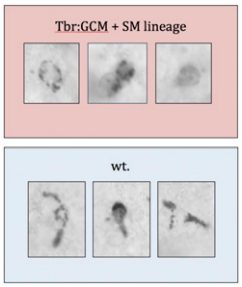

Fig. S4. Comparison of transfated SM cells (*Upper*) and wild-type pigment cells (*Lower*) at 72 hpf.

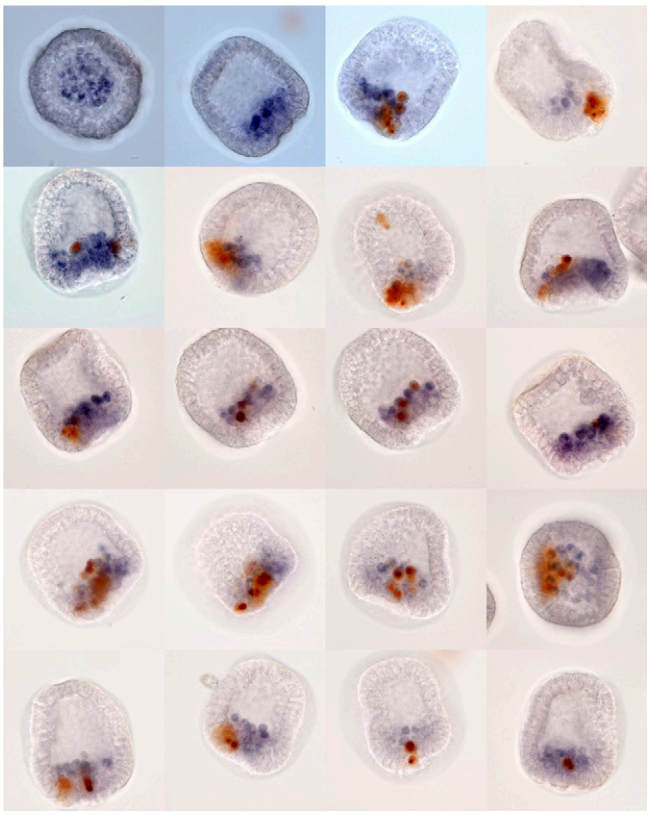

Fig. S5. Alx expression in *tbr*:GCM BAC-injected embryos. *alx*-DIG probe, purple; synthetic *gcm* probe, orange. The leftmost two images in the first row are uninjected control embryos.

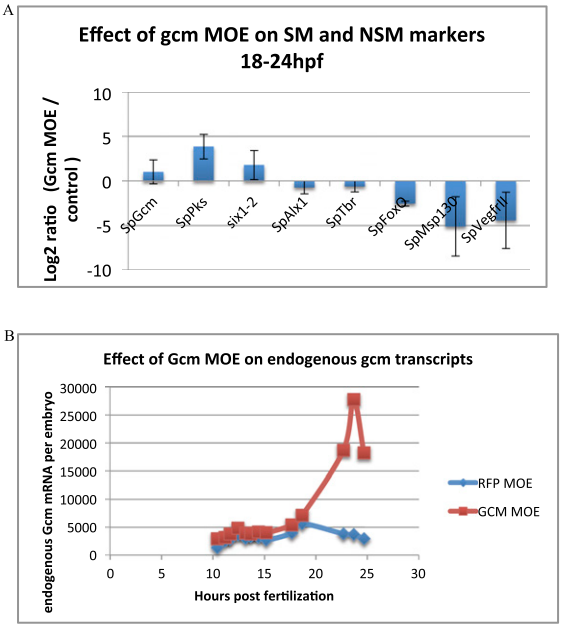

Fig. S6. Effect of *gcm* mRNA overexpression at early mesenchyme blastula stage. Embryos were injected with mRNA of wild-type *gcm*, with control mRNA encoding the *gfp* gene, or with a *gcm* variant with a mutated DNA-binding domain. (*A*) Effect of GCM mRNA overexpression on SM and Non-Skeletogenic Mesenchyme (NSM) markers. Expression is reported as the log2 ratio of mRNA per embryo. Control injection was performed using mRNA encoding a DNA-binding domain mutant of GCM that contains a single amino acid substitution at position 65 of the DNA-binding domain that replaces an asparagine residue with aspartic acid (N65D). The mutant is incapable of binding DNA with wild-type specificity. mRNA were injected at roughly 400,000 copies per embryo. $n = 3$. (*B*) Quantitative PCR time course of the effect of *gcm* mRNA overexpression on endogenous *gcm* mRNA levels. Embryos injected with synthetic *gcm* mRNA were collected at several time points during early development to measure the regulatory effect of GCM on endogenous *gcm* transcription. Injected *gcm* mRNA contained the 5′-UTR of the *tbr* gene and an SV40 3′-UTR, enabling them to be distinguished from endogenous *gcm*. $n = 1$.

Table S1. Expression of *tbr*:RFP and *pks*:GFP detector constructs in the presence or absence of coinjected *tbr*:GCM construct at mid/late gastrulate stage (48 hpf)

	No. of embryos displaying fully transformed SM cells	No. of embryos displaying fully nontransformed SM cells	No. of embryos displaying incompletely transformed SM cells	Total expressing*
Tbr:GCM + Detectors	23 (28%)	54 (67%)	39 (48%)	81 (161)
Detectors only	0	58 (100%)		58 (84)

*Total embryos scored are in parentheses.

Table S2. Quantitation of cell state detectors at 21–24 hpf in the presence of *tbr*:GCM BAC

	ets1:GFP BAC		*tbr:*GFP BAC		*alx*:GFP BAC
	Exp1	Exp2	Exp1	Exp2	Exp1
Without *tbr*:GCM BAC	21.9	142.9	53.0	80.5	61.5
With *tbr*:GCM BAC	8.0	20.4	32.1	47.5	26.5

Data are expressed as copies of GFP mRNA per construct.